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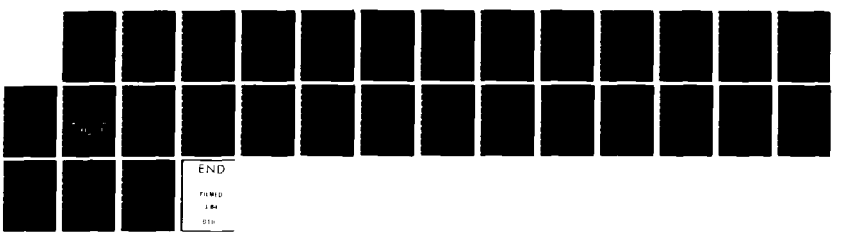

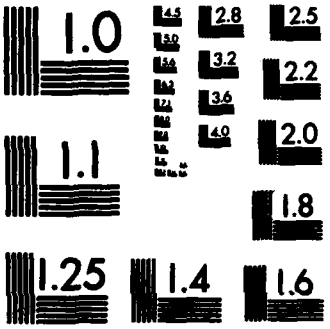

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09 September 1983

THE MOLECULAR TOXICOLOGY OF CHROMATIN

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The chemical (macromolecular) structure and biological function of the eukaryotic cell specific nuclear polymer: polyadenosine diphosphoribose has been investigated in specific cell nuclei isolated from animals (rats) and from human fibroblast cultures. This polymer is formed enzymatically from NAD in eukaryotic nuclei. The polymer was identified for the first time, as

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Item 20. (Continued)

a unique nucleic acid structure which can form helix-helix type non-covalent association between long chain polymers that are on one end covalently bound to non histone proteins, comprising a supramolecular DNA structure associated network system. The influence of differentiation determined by assays in different cell types within one organ and effects of developmental hormones in animals on polyadenosine diphosphoribosylation and the role of the polymer in cell transformation in cell cultures has been studied by focusing on the quantitative and qualitative changes of non histone protein-polyadenosine diphosphoribose adducts in chromatin under controlled experimental conditions. A positive correlation was found between changes (inhibition) in rates of polyadenosine diphosphoribosylation and cellular hypertrophy of cells incapable of DNA synthesis indicating a physiologic control function of the polymer-protein network system in DNA template activity in these cells. It is apparent therefore that the role of poly ADP-ribosylation is different in cells that do not divide as compared to cells with mitotic potential. In cell cultures an S phase specific regulatory role of poly ADP-ribosylation has been identified. Under physiological conditions DNA and poly(ADP-ribose) syntheses run parallel in physiologically functioning cells. When a non toxic but transforming dose of an ultimate carcinogen acts in the S phase the two synthetic functions are uncoupled, and a carcinogen depresses poly ADP-ribosylation, presumably allowing the expression of carcinogen modified DNA sequences (oncogenes?). This dissociation of structural control of DNA by poly ADP-ribosylation can be completely restored by non toxic trace quantities of molecules which at higher concentrations are recognized as inhibitors of poly(ADP-ribose) polymerase (presumably acting as enzyme inducers at low levels). On the basis of these results: a) a new area of gene-expression control has been identified and; b) a biochemical approach to the prevention of environmental chromatin poisoning (by carcinogens, UV or ionizing radiation) became feasible.

Item 20. Abstract:

The chemical (macromolecular) structure and biological function of the eukaryotic cell specific nuclear polymer: polyadenosine diphosphoribose has been investigated in specific cell nuclei isolated from animals (rats) and from human fibroblast cultures. This polymer is formed enzymatically from NAD in eukaryotic nuclei. The polymer was identified for the first time, as a unique nucleic acid structure which can form helix-helix type non-covalent association between long chain polymers that are on one end covalently bound to non histone proteins, comprising a supramolecular DNA structure associated network system. The influence of differentiation determined by assays in different cell types within one organ and effects of developmental hormones in animals on polyadenosine diphosphoribosylation and the role of the polymer in cell transformation in cell cultures has been studied by focusing on the quantitative and qualitative changes of non histone protein-polyadenosine diphosphoribose adducts in chromatin under controlled experimental conditions. A positive correlation was found between changes (inhibition) in rates of polyadenosine diphosphoribosylation and cellular hypertrophy of cells incapable of DNA synthesis indicating a physiologic control function of the polymer-protein network system in DNA template activity in these cells. It is apparent therefore that the role of poly ADP-ribosylation is different in cells that do not divide as compared to cells with mitotic potential. In cell cultures an S phase specific regulatory role of poly ADP-ribosylation has been identified. Under physiological conditions DNA and poly ADP(ribose) syntheses run parallel in physiologically functioning cells. When a non toxic but transforming dose of an ultimate carcinogen acts in the S phase the two synthetic functions are uncoupled, and a carcinogen depresses poly ADP-ribosylation, presumably allowing the expression of carcinogen modified DNA sequences (oncogenes?). This dissociation of structural control of DNA by poly ADP-ribosylation can be completely restored by non toxic trace quantities of molecules which at higher concentrations are recognized as inhibitors of poly(ADP-ribose) polymerase (presumably acting as enzyme inducers at low levels). On the basis of these results: a) a new area of gene-expression control has been identified and; b) a biochemical approach to the prevention of environmental chromatin poisoning (by carcinogens, UV or ionizing radiation) became feasible.

Objectives of research targeted for the 1982-83 period were determined by preceeding work. The fundamental observation (made in 1979-81) that the predominant covalent acceptors of poly(ADP-ribose) are non histone proteins, predicted that the hitherto poorly understood function of nuclear non histone proteins (comprising about 3-500 distinct molecular types) which are assumed to control "specific differentiation related cell function", must be intrinsically linked to the process of poly ADP-ribosylation. It was also assumed that the macromolecular nature of the polymer--that was hitherto thought to represent a "non-structured" (e.g. glycogen-type) polymer--must exhibit specific macromolecular propensities which are likley to determine its nuclear function. It is evident that testing of these two major predictions requires a multi-disciplinary approach. The present report deals with progress made in one year, demonstrating that both predictions could be supported by experimental evidence. First: the macromolecular conformation of poly-(ADP-ribose) was demonstrated to be a highly specific DNA-like helical structure. Second: the enormously complex processes of differentiation and developmental hormone action indeed signalled changes in poly ADP-ribosylation of non histone proteins which appeared to be causally related to cellular responses and more importantly the largely unknown process of neoplastic transformation can now be effectively modified by biochemical interference with the poly ADP-ribosylating system. These experimental results clearly indicate a critical function of poly ADP-ribosylations in cellular biology and are likely to have at least two experimental consequences. One: to open up investigations that can lead to the

clarification of differentiation and neoplasia at a molecular level. Two: a pragmatically feasible control of chromatin toxicity (leading to genetic defects and neoplasia) can be developed against environmental (chemical or radiation) inflicted injuries. The objectives therefore are both molecular structural and cell biological experiments with poly ADP-ribosylations.

Status of Research

I. The resolution of structural parameters of poly(ADP-ribose).

A. First the conformation of the pyrophosphorolytic subunit of the polymer: 2'-(5"-phosphoribosyl)5'-AMP (abbreviated henceforth as PR-AMP) was determined. This specific nucleotide contains a cis-diol in the ribose moiety and it would be expected that it should form the well-known complex with boronic acid. Despite the precise stoichiometry of periodate titration of the cis-diol (shown in Figure 1) indicating its presence as a chemical entity, PR-AMP does not bind to phenyl-boronic acid resin (Figure 2), whereas ADP-ribose (and other cis-diol containing nucleotides) binds. This striking difference was the first chemical evidence that pointed to hitherto unsuspected structural features of this subunit of the polymer. It was therefore logical to assume that the polymer itself must also exhibit unexpected structural features. The hidden nature of the cis-diol in PR-AMP was clarified by the building of computer simulated probabilistic molecular reconstruction, based on known bond angles, X-ray diffraction data, atomic radii, etc.

Figure 1.

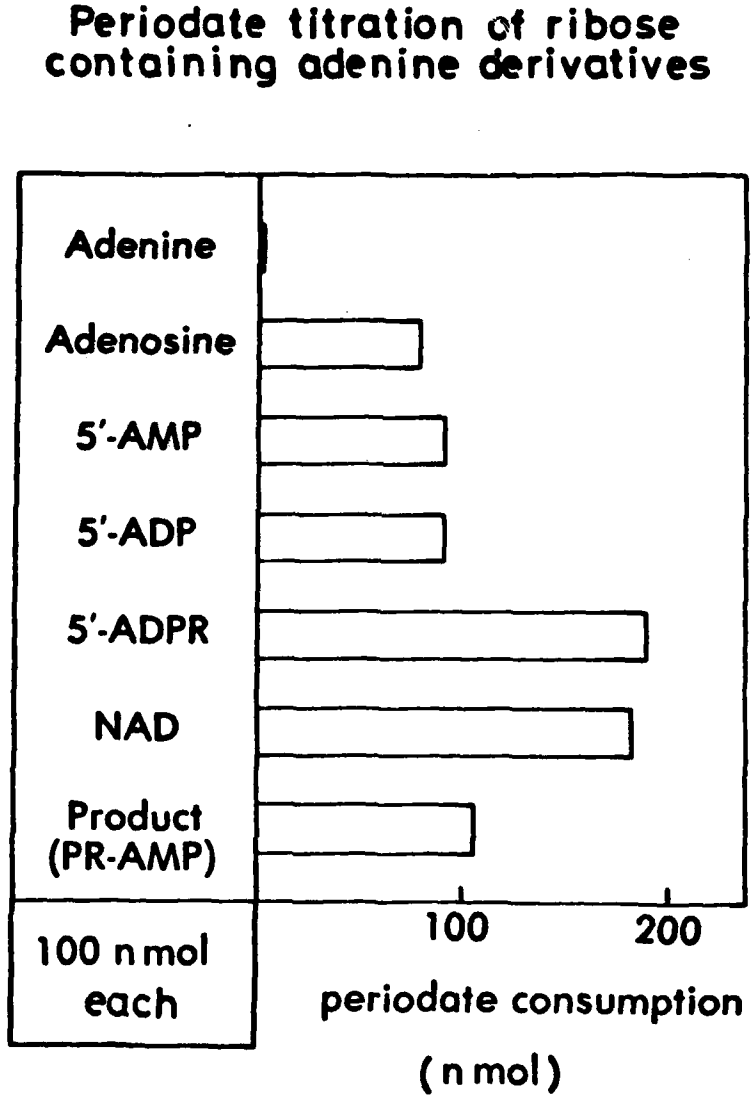
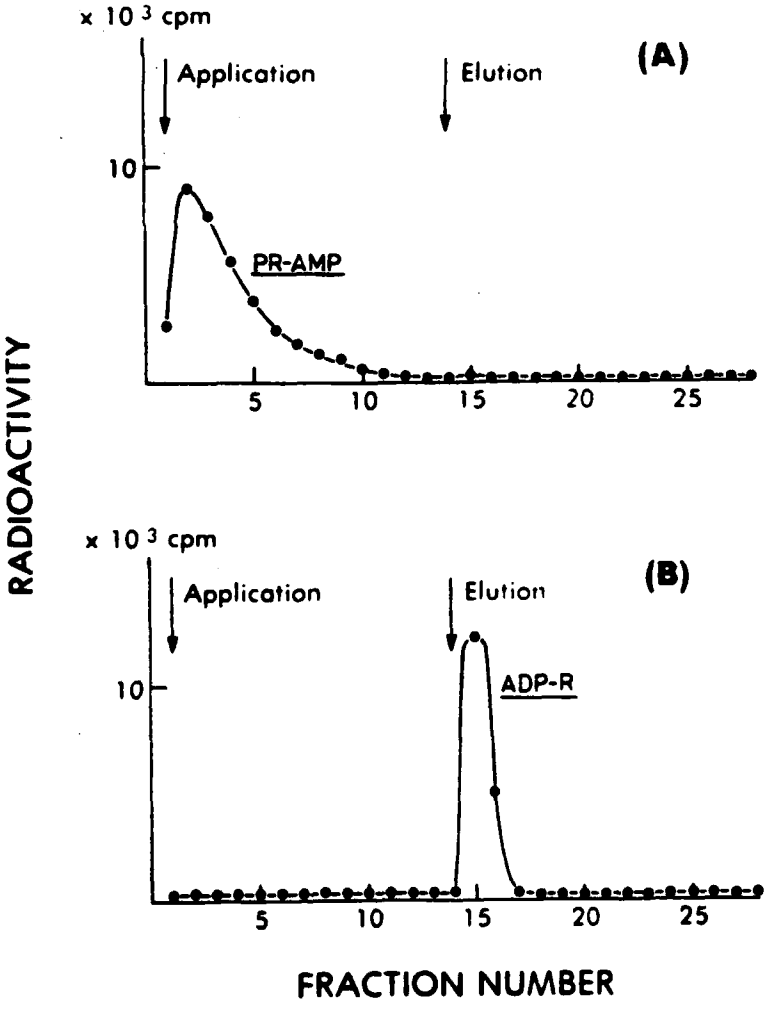
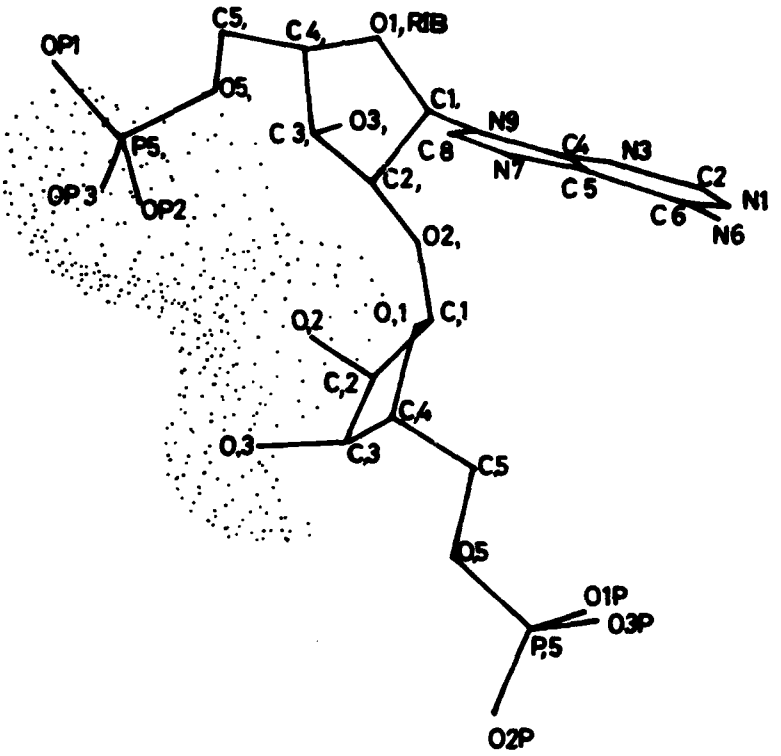

Periodate titration of ribose containing adenine derivatives
Adenine
Adenosine
5'-AMP
5'-ADP
5'-ADPR
NAD
Product (PR-AMP)
100 n mol each
100
200
periodate consumption
(n mol)

Figure 2.


x 10 3 cpm
Application
Elution
(A)
10
PR-AMP
5
10
15
20
25
x 10 3 cpm
Application
Elution
(B)
10
ADP-R
5
10
15
20
25
RADIOACTIVITY
FRACTION NUMBER

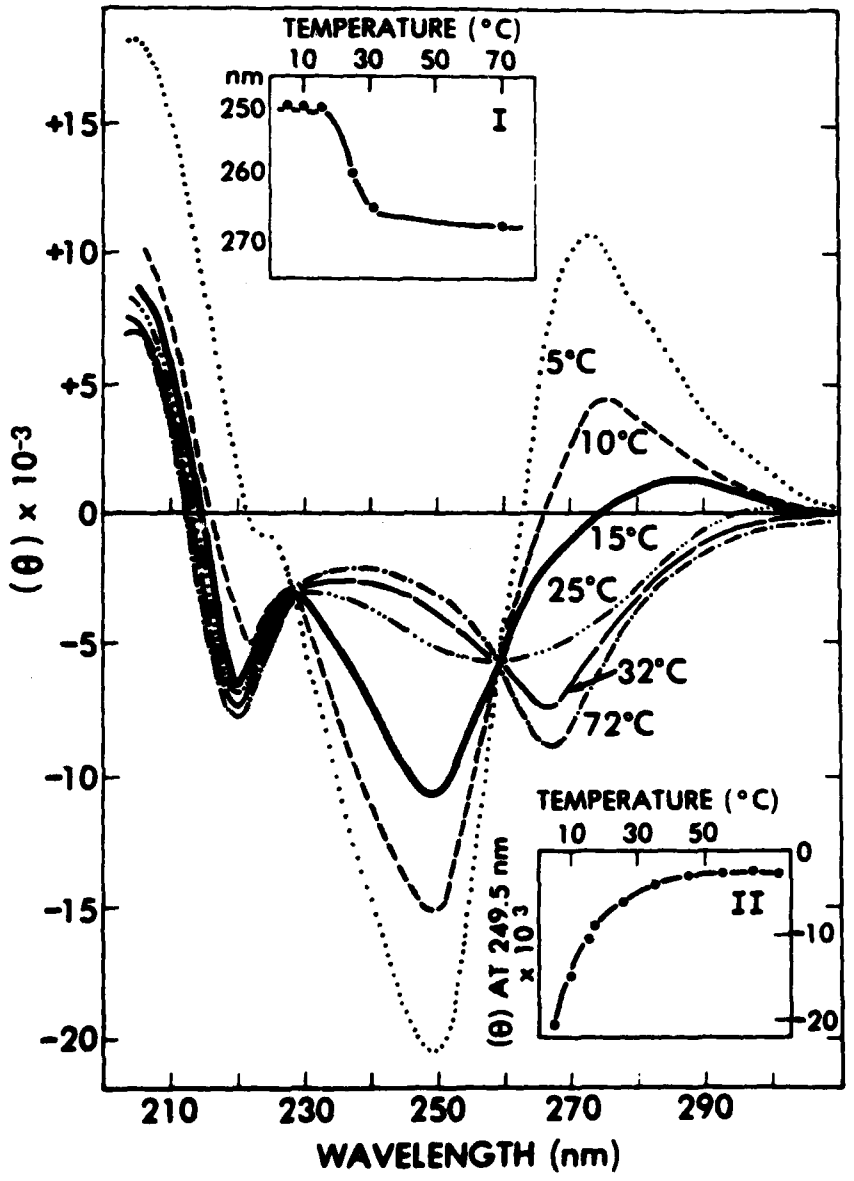
As shown in Figure 3, there is a strong interaction between AMP-phosphate and the cis-diol predicting a significant bend in the polymer backbone, also predicting a lesser acidity of the polymer than calculated from known pK values of phosphoric acid. Details of this work were published in papers 1,2,3.

Figure 3.



B. Macromolecular conformation of poly(ADP-ribose) was determined by direct physicochemical (spectral) analyses. Prerequisite to this work was the development of a large scale preparative technique, suitable for the isolation of highest purity of poly(ADP-ribose) which contains no traces of other nucleic acids that would invalidate spectral studies. The previously reported methods were found unsuitable, therefore a new technique was developed based on affinity chromatography. Details were published in reference 4 and pure polymers of short, medium, and long chain length were prepared in several mg/batch scales. More recently (A. Hakam and E. Kun) this technique was improved to yield 3-400% more than reported in reference 4. The most unambiguous optical identification of macromolecular conformations, in circular dichroism (CD), and as shown in Figure 4, molar assymetry (θ) plotted against wave length (nm) in case of long ($n>32$) polymers gave characteristic temperature dependent CD spectra, reminiscent of DNA. Details of these analyses were published in reference 4.

Figure 4.


TEMPERATURE (°C)
10 30 50 70
nm
250
260
270
I
+15
+10
+5
0
-5
-10
-15
-20
(θ) x 10-3
5°C
10°C
15°C
25°C
32°C
72°C
TEMPERATURE (°C)
10 30 50
(θ) AT 249.5 nm x 10-3
0
-10
-20
II
210 230 250 270 290
WAVELENGTH (nm)

Further studies revealed a salt concentration dependent melting characteristic as illustrated in Figures 5 and 6, consistent with helical structure. A new mathematical relationship between $A_{280}/_{260}$ and θ was discovered, allowing calculation of molar assymetry on the basis of UV analysis alone. This and exciton transfer computation strongly support a helical conformation of physiologically occurring long chain poly(ADP-ribose) as discussed in detail in reference 5.

Figure 5.

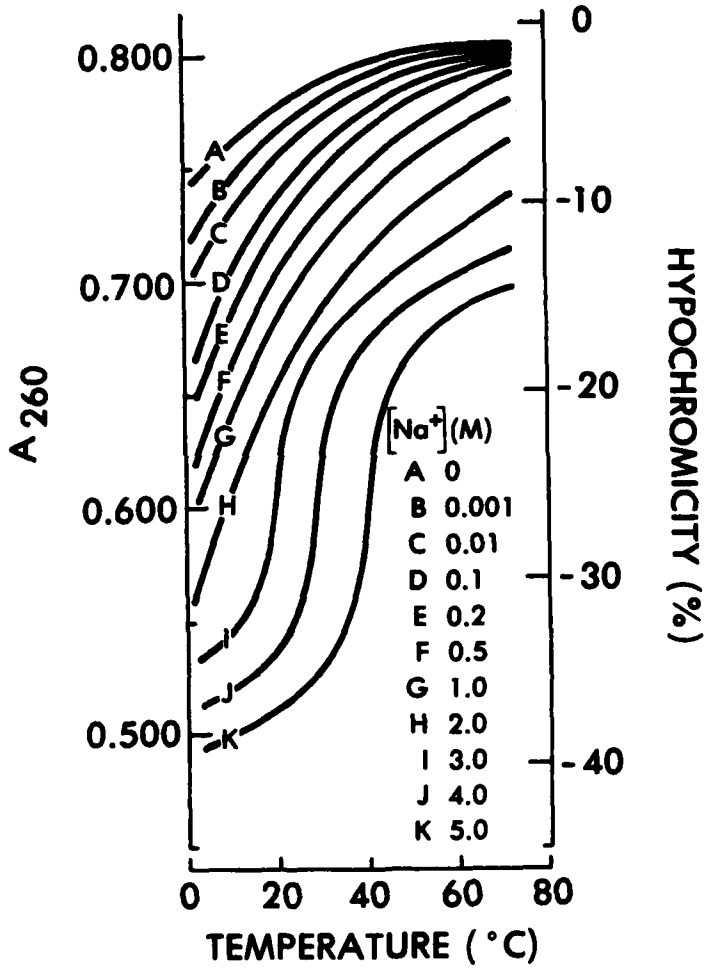
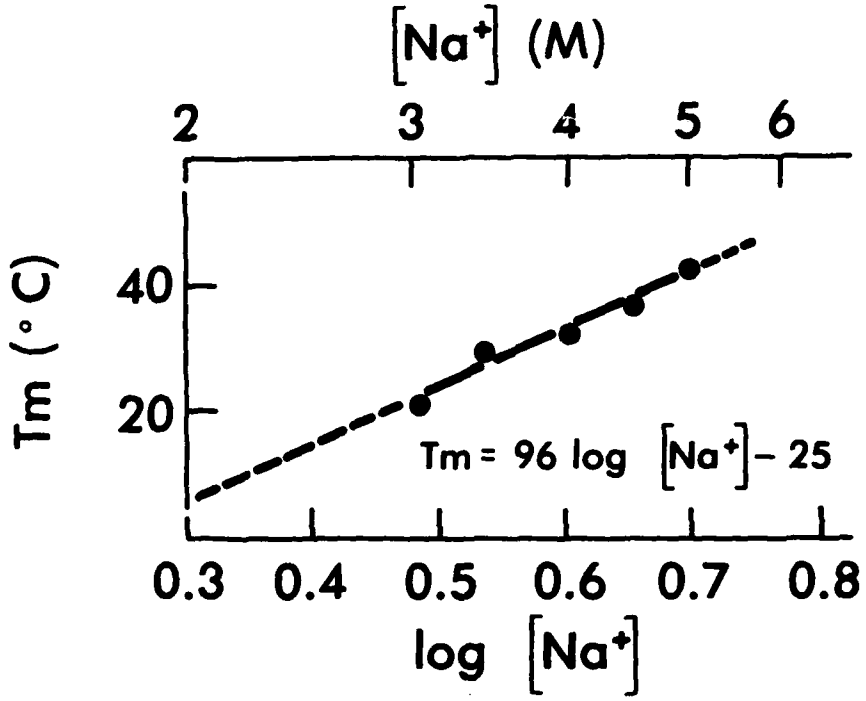


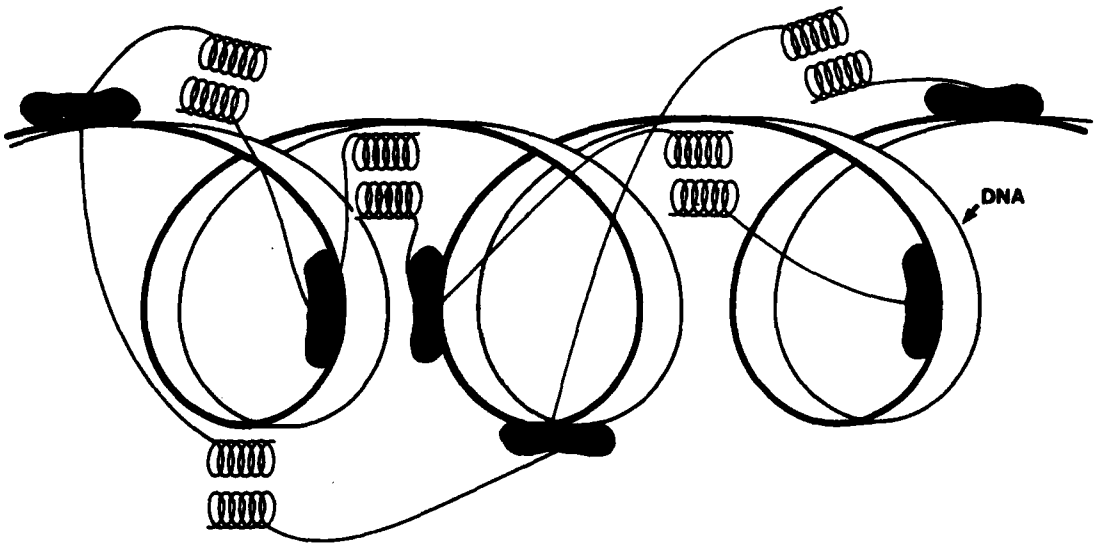
Figure 6.

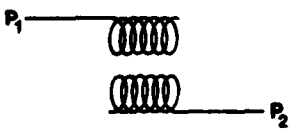


This discovery is of paramount importance in defining the structure of poly ADP-ribosylated non histone proteins in chromatin in relationship to double stranded DNA. Since we find (references 2,3) that non histone proteins that serve as polymer acceptors are tightly bound to DNA it is plausible that the covalently bound helical poly(ADP-ribose) chains can interact with each other by helix-helix interaction (forming probably double helices). This interaction force is predictably quite large (compare to double helical DNA) thus a network as illustrated in Figure 7 is plausible.

Figure 7.

POLY(ADP-RIBOSE)-NHP NETWORK IN CHROMATIN





Helix-helix interaction between poly(ADP-ribose) chains attached to two different NHP (P_1, P_2)

The following predictions can be made:

a) The supra-structure of chromatin (consisting of DNA + proteins) is most probably held in a specific shape by DNA associating non histone (NHP) proteins by way of a helix-helix interactions-stabilized network system (Figure 7). This comprises a structural control superceeding nucleosome structures (that are "inside" this network) and the well-known karyological nuclear changes that accompany cell cycle, cell-division etc., and were seen by microscopists since the beginning of this century now can be explained on a macromolecular basis.

b) It follows that if poly(ADP-ribose) plays the role of a structural coenzyme of the nucleus (reminiscent of the coenzymatic function of NAD from which this polymer is made) the enzyme poly(ADP-ribose) polymerase by its activity (or quantity) must regulate nuclear supramolecular structure, thus a catalytic control of supramolecular conformation of chromatin exists.

c) It also follows that the molecular mechanism of action of poly-(ADP-ribose) is unlikely to be explained by a single target protein interaction, but more probably by a concerted action on several critical DNA-binding proteins (which may be enzymes or DNA regulating proteins). It is of profound significance that despite this large complexity we obtained well defined quantitative correlations between poly ADP-ribosylations and cell biological responses, suggesting that the enzyme poly(ADP-ribose) polymerase must be highly regulated (by as yet unknown systems) and that concerted polymer attachment of specific NHP-s to DNA is not random but selective and permit only a limited number of functionally distinct configurations.

The elucidation of poly(ADP-ribose) polymerase control in chromatin clearly comprises a significant future project that is likely to explain in molecular terms cellular pleiotropic responses that are well-known to exist, but their relationship is missing.

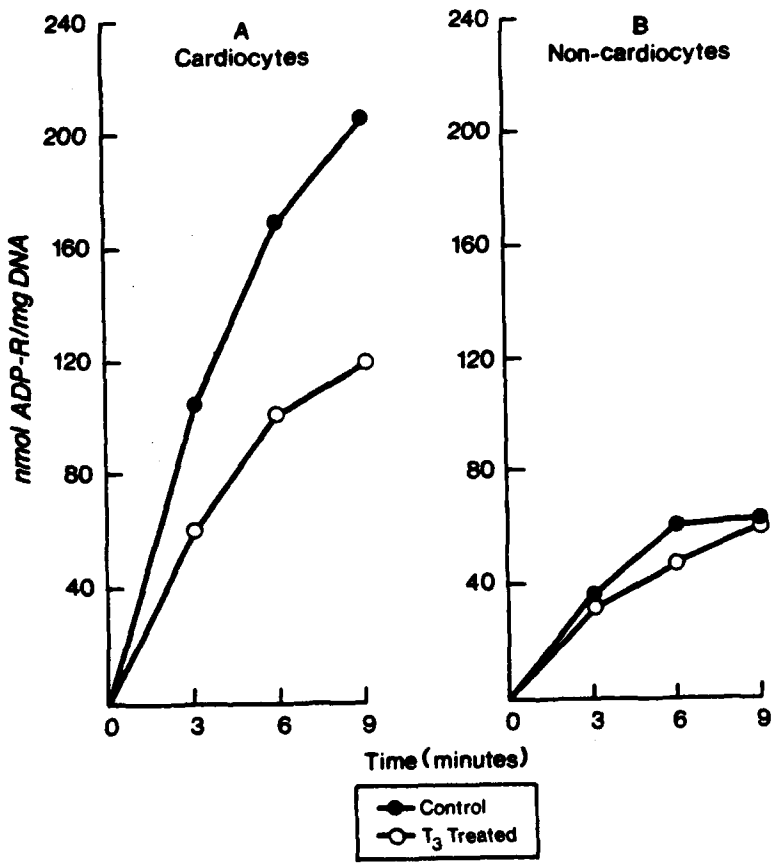
Summary

Direct physicochemical evidence shows a specific nucleic acid-like structure of poly(ADP-ribose) and this structure is capable of predicting a macromolecular control of chromatin conformation. It is known that genes in isolated DNA are located discontinuously (interjection of non-expressed introns, regulatory segments, etc.). One teleological explanation for this apparent discontinuity seen in isolated DNA is that the suprastructure of DNA actually accomodates molecular vicinity of DNA segments in supercoiled DNA so that the discontinuity seen in linear DNA strands actually may not exist in the supercoiled form. It follows that specific topology of supercoiled DNA can determine (regulate) the continuous (active) or disrupted (inactive) reading of genetic information. Our results indicate that the maintenance of DNA superstructure may be controlled by the NHP-poly(ADP-ribose) network system (Figure 7), implying that the positioning of DNA-segments belonging to one particular gene can be controlled by the NHP-polymer system. This mechanism identifies the NHP-poly(ADP-ribose) system as a critical gene expression controlling device (see also reference 9).

II. Biological correlations of poly ADP-ribosylation processes in eukaryotes.

A. Animal experimentation, hormonal and developmental dependent responses. If--according to the mechanism proposed in I--the structural control of chromatin by the poly ADP-ribosyl-NHP system governs the expression of complex functions (e.g. differentiation, hormone-mediated responses and cell transformation) it would be expected that quantitative (and qualitative) changes in the NHP-poly(ADP-ribose) should coincide with phenomena representing differentiation or transformation. The cardiac tissue model is particularly suitable for this study because it provides experimental separation of non-dividing (cardiocyte) and dividing (non-cardiocyte) nuclei from the same organ. Therefore differentiation dependent controls can be determined in the same system. As detailed in references 6,7,8,9, there is a marked difference between poly ADP-ribosylation in cardiocyte and non-cardiocyte nuclei, as shown in Figure 8, and the developmental hormone triiodothyronine (T_3) selectively depresses poly-(ADP-ribose) polymerase in cardiocyte nuclei only (reference 9), confirming the basic assumption that the biological regulation of chromatin by the NHP-poly(ADP-ribose) network system (Figure 7) indeed is determined by the ontogenic history of cell types.

Figure 8.


A
Cardiocytes
B
Non-cardiocytes
240
200
160
120
80
40
nmol ADP-R/mg DNA
0
3
6
9
Time (minutes)
Control
T3 Treated

As discussed in detail in references 6,7,8,9, selectivity of control is coincidental with variation in the groups of polymer acceptor proteins that are dependent on the cell system studied (references 6,9). The physiological implications in the regulation of cell hypertrophy (enlargement without cell division) are discussed in reference 8, where we show that inhibition of poly ADP-ribosylation coincides with increased (de-repressed) rates of RNA and protein synthesis. Further work is concerned with chemical and immunological identification of DNA controlling NHP-s and the effects of poly ADP-ribosylation on the binding affinities to selective DNA restriction fragments that should be deterministic in various template functions of DNA.

Summary: Physiological evidence is accumulating that is in accord with the predicted chromatin regulatory function of NHP-poly(ADP-ribose) adducts in animal organisms. The physiological process of stress induced cellular hypertrophy coincides with selective inhibition of poly(ADP-ribose) polymerase in cell types that lost their capacity to divide.

B. Cell culture models of control by poly(ADP-ribose)-NHP-s.

The seemingly even more complex problem of cell transformation, which in a biological sense is a tradeoff for differentiation, yielded unexpectedly, unambiguous results in the human fibroblast cell culture model of Milo.

Present views on neoplastic transformation appear to focus on the problem of proto-oncogen→ oncogene transformation and as yet unknown mechanisms are recognized that may govern the expression of oncogenes (see: Duesberg,P.H., Nature, 1983, Vol.304, July 21, p.219). One of the major drawbacks of models employed by workers in this field is the almost

exclusive use of 3T3 cells, that are already transformed (they are immortal), therefore none of the conclusions drawn from this work can be applied to normal mortal differentiated cells. This fault is presently becoming apparent and a revision of the entire field will have to follow (see Duesberg). In all models proposed the actual mechanism of "oncogene-expression" is routinely glossed over implying unknown molecular processes in chromatin. The model shown here in Figure 7, has the advantage of directly explaining chromatin-structure related activation or inhibition of gene expression and we proceeded to test the direct prediction: if the structural model is dependnet on poly ADP-ribosylation and the model does indeed explain gene activation, then neoplastic transformation should be modifiable by interferring with poly ADP-ribosylation. We employed specific enzymatic probes of poly ADP-ribosylation to test its role in transformation (see references 2 and 10).

Cell Cycle Dependence

It is known that poly (ADP-ribose) polymerase activity exhibits cell cycle dependent oscillation, maxima coinciding with S phase. The biological significance of this oscillatory kinetics has remained unexplained. The model shown in Figure 7 has the capability of providing a mechanistic interpretation of the oscillation of poly ADP-ribosylation within the cell cycle. In S phase critical events of synchronously appearing DNA replication forks (leading to precisely one replication) have to be regulated in a highly deterministic manner. Suprastructural DNA conformation changes are highly probable events that may accomplish the required DNA

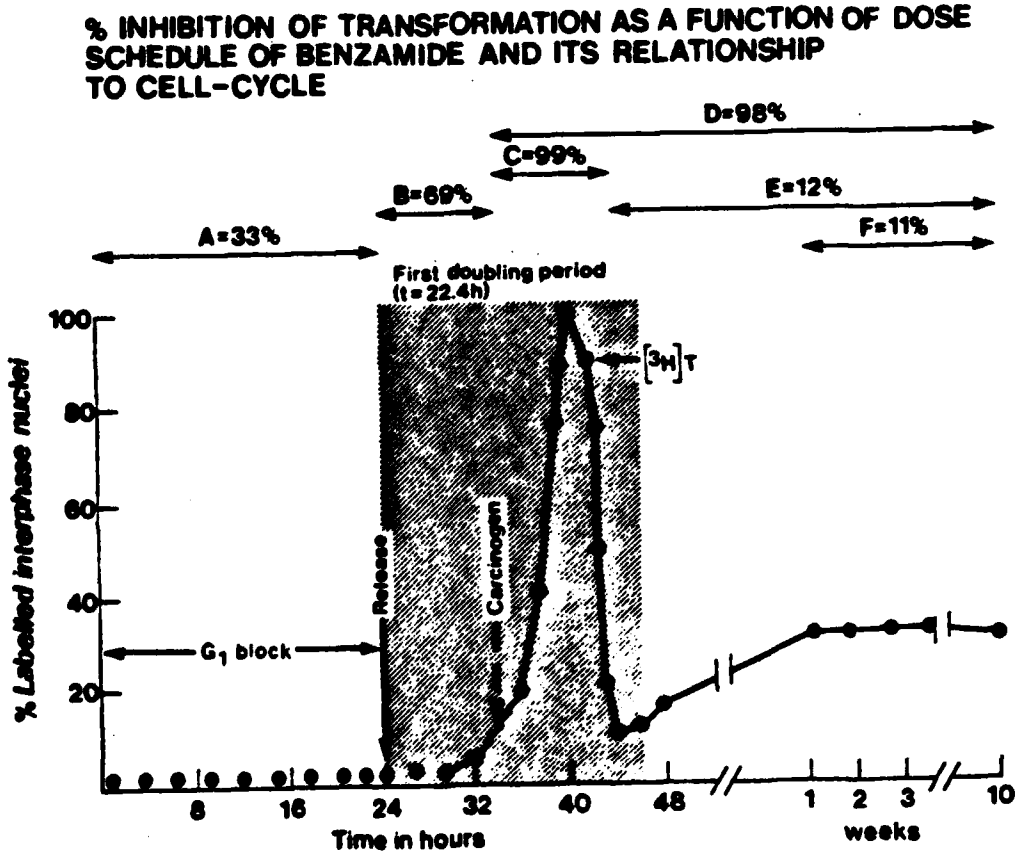
changes in S phase, preparing for mitosis. The oscillation of the poly-(ADP-ribose) NHP network system seems therefore a feasible candidate for this complex control.

In our earlier studies (JBC 256, 7800-7805, 1981) the critical observation was made that in an early exposure time to dimethylnitrosamine significant increase in hepatic poly(ADP-ribose)-NHP-s occurred. Subsequently we found (reference 11) that liver regeneration itself, without carcinogens, accomplishes a similar increase in poly(ADP-ribose)-NHP-s. Since the common denominator between the two experimental conditions is a disproportional augmentation of cells in S phase (in the carcinogen treated conditions dimethylnitrosamine at low doses acts also as an S phase augmenting mitogen) it seemed plausible to explain the hitherto obscure "promoter" state as an increased incidence of S (which is known to be more susceptible to carcinogenic factors than any other cell cycle phase). It follows that a direct experimental testing of the predicted role of cellular S phase in transformation should be undertaken.

Milo's human fibroblast model proved to be a straight-forward test system. As discussed in reference 10, transformation of normal fibroblasts critically depends on the narrow window of exposure of synchronized cells in the early S phase to ultimate carcinogens (or UV or γ-radiation) precisely coinciding with the physiological augmentation of poly ADP-ribosylation in normal S phase without carcinogens in animal models (compare to regeneration, cf. reference 11). We argued: if poly ADP-ribosylation augmentation in S phase is physiological and clearly is a synchronous event with DNA synthesis (in S phase) then carcinogens should by some mechanism disrupt this

synchrony. As described in reference 10, highly effective ultimate carcinogens indeed dissociate poly ADP-ribosylation from DNA synthesis by depressing the former. Augmentation of poly ADP-ribosylation by specific agents (recognized as poly(ADP-ribose) polymerase inhibitors) by reconstructing physiological rates of poly ADP-ribosylation should then prevent transformation. The S phase specificity of these events is illustrated in Figure 9 which also shows the effectivity of benzamide (a potent probe of poly(ADP-ribose) polymerase) as transformation preventor (the horizontal lines A to F indicate the % transformation - inhibition as a function of kinetics of exposure to non toxic doses of this agent).

Figure 9.



It should be pointed out that the role of an enzyme inhibitor as an enzyme augmenting inducing agent, the latter effect playing the critical role in transformation prevention may seem difficult to grasp at first approximation. Biochemical argument would suggest that the enzyme inhibition ought to be the transformation inhibitory mechanism. However this reasoning would suggest that a physiological enzymatic reaction: poly(ADP-ribose) polymerase plays an unphysiological (transformation promoter) role, which on the basis of evolutionary logic seems unreasonable, unless the genome of poly(ADP-ribose) polymerase itself is a proto-oncogene, a less probable point that is being investigated. Our present evidence favors the enzyme inducer function of antitransforming agents as a critical mechanism in transformation prevention. At higher concentrations these agents also inhibit poly(ADP-ribose) polymerase but this may be a toxic side reaction. The observed 20-30% inhibition of the polymerase by benzamide has apparently no measurable unphysiological consequences and may reflect a physiological safety margin of enzyme activity. As yet unpublished experiments (Kun, Kirsten, Milo, and Hasam) show that we now posess molecules that are anti-transformers at low concentrations that do not inhibit poly(ADP-ribose) polymerase. Discovery of these antitransformers however was made on the basis of their enzyme inhibitory property at much higher concentrations providing an excellent method to search for antitransformers. Benzamide happens to be effective at an intermediary level, where partial inhibition (20-30%) of the polymerase coincides with the concentration of benzamide that also serves as an inducer and antitransformer. Extension of this highly important mechanism involving the identification of the poly(ADP-ribose) polymerase gene and the mode of its activation is subject of future investigations.

The transformation preventing effect of a presently most extensively studied agent: benzamide is shown in Table I, and the quantitative changes of poly(ADP-ribose)-NHP-s during normal S phase after exposure to carcinogen or benzamide alone or to simultaneous exposure, resulting in transformation prevention is shown in Table II.

Table I.

Inhibition by benzamide of carcinogen induced cell transformation as determined by colony counts on soft agar. Both carcinogens and benzamide were present in RCF_1 concentrations.

No.	No. of Experiments	Experimental Conditions	No. of transformed cell colonies per 10^5 cells.
1.	5	a. methylazoxy methanol acetate (7.0 μM)	758 (± 58)
	5	b. (a.) + benzamide	1 (± 0.3)
2.	2	a. N-methyl,N'-nitro N-nitrosguanidine (0.7 μM)	39 (±6)
	2	b. (a.) + benzamide	0
3.	2	a. 7β, 8α-dihydroxy-9α-10α epoxy-7,8,9,10-tetrahydrobenzpyrene (0.33 μM)	26 (±2)
	2	b. (a.) + benzamide	0
4.	2	a. β-propiolactone (28 μM)	28 (± 3)
		b. (a.) + benzamide	0
5.	1	a. 1,1-dimethyl hydrazine (167 μM)	103 (± 9)
	1	b. (a.) + benzamide	1 (± 0.2)
6.	2	a. 3-hydroxyl-1-propane sulfonic acid γ-sultone (122 μM)	43 (± 5.5)
	2	b. (a.) + benzamide	1.0 (± 0.3)
7.	5	no additions	0
8.	5	benzamide	0

Table II.

Distribution and quantities of total amounts, phenol and H_2O soluble protein-poly(ADP-ribose) adducts.

	Experimental Conditions	Time of reaction in minutes	Protein-bound ADP-ribose in p mol/10^6 cells		
			total	phenol soluble	H_2O soluble
1.	G block	20	203	45	157
		40	324	35	289
2.	S phase	20	413	126	287
		40	868	13	854
3.	S + benzamide	20	207	67	140
		40	294	29	265
4.	S + MAMA	20	279	63	214
		40	348	19	329
5.	S + benzamide + MAMA	20	564	245	318
		40	905	15	888

Each value is the mean of 3 analyses with SD of $\pm$ 20%.

Summary and Prospectus

From the present state of experimental work direct evidence of a supramolecular structural control of chromatin emerges. The poly(ADP-ribose)-NHP network system provides a plausible model, that connects metabolic (NAD/NADH ratio-dependent) and epigenetic signals, resulting in almost quantized regulation of critical events in the cell cycle, that can explain conformation dependent "opening up" or "occlusion" of DNA segments to serve as templates for DNA or RNA synthesis. Poly(ADP-ribose) polymerase enzyme (a protein which we have recently obtained in pure form and antibodies against it are being generated in rabbits at present) plays an absolutely critical role in this regulatory system and much future work is required to sort out detailed molecular mechanisms. As a consequence of the newly identified macromolecular chromatin system, the cell culture model provided the first evidence of its real biological significance. This work is also in its early phase. However it is predictable that a planned intervention of this system is likely to lead to both new theoretical and practical results.

1982-1983 Publications:

1. Minaga,T., McLick,J., Pattabiraman,N., and Kun,E. (1982) "Steric Inhibition of Phenylboronate Complex Formation of 2'(5"-phosphoribosyl)-5'-AMP. J. Biol. Chem. 257, 11942-11945.

2. Kun,E., Minaga,T., Kirsten,E., Jackowski,G., Peller,L., Marton,L., Oredsson,S.M., and Milo,G. (1982) "Regulation of Chromatin Function by Polyadenosine Diphosphoribosylation." XII Steenbock-Lilly Symp. University of Wisconsin, June 8-11, 1982. (Proceedings, Elsevier Publ.).

3. Kun,E., Minaga,T., Kirsten,E., Jackowski,G., McLick,J., Peller,L., Oredsson,S.M., Marton,L., Pattabiraman,N., and Milo,G. (1983) "Biochemical Basis of the Regulatory Role of Polyadenosine Diphosphoribose ," in Advances in Enzyme Regulation (Pergamon Press, London) Vol.21, pp.177-199.

4. Minaga,T. and Kun,E. (1983) "Probable Helical Conformation of Polyadenosine Diphosphoribose: Evidence Indicating Secondary Structure." J. Biol. Chem. 258, 725-730.

5. Minaga,T. and Kun,E. (1983) "Probable Helical Conformation of Poly(ADP-ribose. The Effect of Cations on Spectral Properties." J. Biol. Chem. 258, 5726-5730.

6. Jackowski,G. and Kun,E. (1982) "The Influence of Triiodothyronine on Polyadenosine Diphosphoribose Polymerase and RNA Synthesis in Cardiocyte Nuclei." J. Mol. Cell. Cardiol. 14, Suppl.3, 65-70.

7. Jackowski,G., Romaschin,A.D., and Kun,E. (1982) "Age Dependent Selective Effects of Hydrocortisone and Aldosterone on the Polyadenosine Diphosphoribose Metabolism of Isolated Cardiocyte Nuclei." Biochem. Internat. 4, 17-24.

8. Jackowski,G., Heymann,M.A., Rudolph,A.M., and Kun,E. (1982) "Cell Specific Response of Cardiac Poly(ADP-R) Synthesis to Circulatory Stress." Experientia, 38, 1068-1069.

9. Jackowski,G. and Kun,E. (1983) "The Effect of In Vivo Treatment with Triiodothyronine on the In Vitro Synthesis of Protein-Poly(ADP-ribose) Adducts by Isolated Cardiocyte Nuclei and the Separation of Poly(ADP-ribosylated Proteins by Phenol Extraction and Electrophoresis." J. Biol. Chem.(to appear in October issue).

10. Kun,E., Kirsten,E., Milo,G.E., Kurian,P., and Kumari,H.L. (1983) "Cell Cycle Dependent Intervention by Benzamide of Carcinogen Induced Neoplastic Transformation and In Vitro Poly(ADP-ribosyl)ation of Nuclear Proteins in Human Fibroblasts." Proc. Natl. Acad. Sci. USA (in press).

Publications Continued.

11. Kirsten,E., Minaga,T., and Kun,E. (1982) "Coincidence of Subnuclear Distribution of poly(ADP-ribose) Synthetase and DNA Polymerase in Nuclei of Normal and Regenerating Liver." FEBS Letts. 139, No.1, 117-120.

Invited Speaker to the Following Sympoisa:

Conference on Post-Translation Covalent Modification of Proteins for Function. Oklahoma Medical Research Foundation. Oklahoma City, November 8-10, 1982.

7th International Symposium of Metabolic Interconversion of Enzymes, Avila, Spain, May 16-18, 1983.

21st International Symposium of the Regulation of Enzyme Activity and Synthesis in Normal and Neoplastic Tissue. Indiana University, School of Medicine, Indianapolis, October 4-5, 1982.

Invited Seminar Speaker to:

Columbia University, New York
Hoffmann-LaRoche Co. (Basel,Switzerland)
Basel Institute for Immunology
Los Alamos National Laboratory, New Mexico
Johns Hopkins University School of Medicine, Baltimore

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